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The following listing of claims will replace all prior versions, and listings, of claims in this application.

## **LISTING OF THE CLAIMS**

- 1. (Currently Amended) A process for producing transglutaminase having an enzymatic activity comprising:
  - (a) incubating a denatured transglutaminase in an acidic aqueous medium;
  - (b) diluting the denatured transglutaminase in the acidic aqueous medium by about 5-fold to about 400-fold; and
  - (b) (c) adjusting the pH of said aqueous medium to a neutral pH by adding an alkali to said aqueous medium.



- 2. (Original) The process as claimed in claim 1, wherein the aqueous medium further comprises a reducing agent.
- 3. (Original) The process as claimed in claim 2, wherein the reducing agent is selected from the group consisting of dithiothreitol, 2-mercaptoethanol, and tris-(2-carboxyethyl)phosphine.
- 4. (Original) The process as claimed in claim 1, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.
- 5. (Original) The process as claimed in claim 4, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

- 6. (Original) The process as claimed in claim 4, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.
- 7. (Original) The process as claimed in claim 1, wherein the aqueous medium in step(a) further comprises a protein denaturant.
- 8. (Original) The process as claimed in claim 7, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.
- 9. (Currently Amended) The process as claimed in claim 7, wherein the transglutaminase concentration is from at least 40 mg/ml and the protein denaturant concentration is from 4 to 10 M.
- 10. (Original) The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 2 to 7.
- 11. (Original) The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 3 to 5.
- 12. (Original) The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 3.5 to 4.5.

13. (Currently Amended) The process as claimed in claim 1, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted at least 5-fold.

14. (Currently Amended) The process as claimed in claim 1, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted at least 10-fold.

15. (Currently Amended) The process as claimed in claim 1, wherein preceding step

(b), the acidic aqueous medium of said denatured transglutaminase is diluted from at

least 50-fold to 400-fold.

16. (Original) The process as claimed in claim 1, wherein said incubation is performed at not more than 15 C.

17. (Original) The process as claimed in claim 1, wherein said incubation is performed from 3 to 10 C.

18. (Original) The process as claimed in claim 1, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted to a concentration of not more than 10 mg/ml.

19. (Original) The process as claimed in claim 1, wherein said neutral pH is from 5.8 to 8.5.

- 20. (Original) The process as claimed in claim 1, wherein said neutral pH is from 6 to 7.
- 21. (Currently Amended) The process as claimed in claim 1, wherein in step (b)(c), the aqueous medium further comprises an accelerator for forming a higher-order native-state transglutaminase structure having enzymatic activity.
- 22. (Original) The process as claimed in claim 21, wherein the accelerator is selected from the group consisting of an inorganic salt, an organic salt, an amino acid salt, a polyol, an organic solvent, and a surfactant.



- 23. (Currently Amended) The process as claimed in claim <u>22</u> <del>21</del>, wherein the <u>accelerator is an inorganic salt accelerator, which</u> is selected from the group consisting of calcium chloride and strontium chloride.
- 24. (Currently Amended) The process as claimed in claim 23 21, wherein the inorganic salt accelerator concentration is from 0.01 to 10 mM.
- 25. (Currently Amended) The process as claimed in claim 22 21, wherein the accelerator is an organic salt accelerator, which is selected from the group consisting of sodium acetate and sodium propionate.
- 26. (Currently Amended) The process as claimed in claim 25 21, wherein the organic salt accelerator concentration is from 0.1 to 2 M.

- 27. (Currently Amended) The process as claimed in claim <u>22</u> <del>21</del>, wherein the <u>accelerator is an amino acid salt accelerator and</u> is arginine hydrochloride.
- 28. (Currently Amended) The process as claimed in claim 27 21, wherein the amino acid salt accelerator concentration is from 0.1 to 2 M.
- 29. (Currently Amended) The process as claimed in claim <u>22</u> <del>21</del>, wherein the <u>accelerator is a polyol accelerator and is polyethylene glycol.</u>



- 30. (Currently Amended) The process as claimed in claim 2129, wherein the polyol accelerator concentration is from 1 to 10%.
- 31. (Currently Amended) The process as claimed in claim 22 21, wherein the accelerator is an organic solvent accelerator which is selected from the group consisting of DMSO and DMF.
- 32. (Currently Amended) The process as claimed in claim <u>31</u> <del>21</del>, wherein the organic solvent accelerator concentration is from 10 to 40%.
- 33. (Currently Amended) The process as claimed in claim <u>22</u> <del>21</del>, wherein the <u>accelerator is a surfactant and is CHAPS.</u>
- 34. (Original) The process as claimed in claim 21, wherein the surfactant concentration is from 1 to 50 mM.

- 35. (Currently Amended) The process as claimed in claim 1, further comprising:(c) centrifugating the aqueous medium of (c)(b).
- 36. (Currently Amended) An isolated transglutaminase obtained by the process of claim 1, which has an intermediate structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.
- 37. (Currently Amended) The process as claimed in claim 1, wherein step (c)(b) further comprises incubating the aqueous medium for more than 1.5 hours subsequent to adjusting the pH to a neutral region.
- 38. (Currently Amended) A process for producing transglutaminase having an enzymatic activity, which comprises subjecting denatured transglutaminase to the following steps (a) and (b):
  - (a) a step for forming an intermediate <u>transglutaminase</u> structure <u>in which said</u> transglutaminase in the denatured state is incubated in an aqueous medium under acidic conditions; and
  - (b) a step for forming a higher-order native-state structure exhibiting enzymatic activity by adjusting the pH of the aqueous medium having said intermediate structure to a neutral pH.

Claims 39 and 40 (cancelled).

- 41. (Original) The process as claimed in claim 38, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.
- 42. (Original)The process as claimed in claim 41, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.
- 43. (Original)The process as claimed in claim 41, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.

Claims 44-71 (Cancelled).

- 72. (Original) The process as claimed in claim 38, further comprising:
- (c) a step for separating inactive enzyme(s) as aggregate(s) by centrifugation.
- 73. (Currently Amended) An isolated transglutaminase obtained by the process of claim 38, which has an intermediate structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.

Claim 74 (Cancelled).

75.(Original) A transglutaminase comprising the following properties (a) to (d):

(a) specific activity of 15 to 25 U/mg provided through measurement of transglutaminase activity by the hydroxamate method;

- (b) a molecular ellipticity which is 30 to 70% of that of the native state in a CD spectrum of a near ultraviolet region;
- (c) a molecular weight of 36,000 to 40,000 as measured by SDS-polyacrylamide gel electrophoresis; and
- (d) lower mobility than that of a native state in native-polyacrylamide gel electrophoresis with a His-Mes buffer system of pH 6.1.
  - 76. (New) A food comprising the transglutaminase of Claim 36.
  - 77. (New) The food of Claim 76, which is a jelly, yogurt, cheese or meat.
  - 78. (New) A toiletry comprising the transglutaminase of Claim 26.
  - 79. (New) A food comprising the transglutaminase of Claim 73.
  - 80. (New) The food of Claim 79, which is a jelly, yogurt, cheese or meat.
  - 81. (New) A toiletry comprising the transglutaminase of Claim 73.
  - 82. (New) A food comprising the transglutaminase of Claim 75.
  - 83. (New) The food of Claim §2, which is a jelly, yogurt, cheese or meat.
  - 84. (New) A toiletry comprising the transglutaminase of Claim 75.
- 85. (New) In a method of producing a food comprising a transglutaminase, the improvement comprising producing the transglutaminase according to the process of Claim 1.
- 86. (New) In a method of producing a food comprising a transglutaminase, the improvement comprising producing the transglutaminase according to the process of Claim

38,